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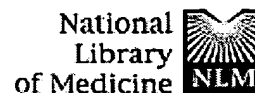
Dipartimento di Sanita Pubblica e Biologia Cellulare, Universita degli Studi di Roma Tor Vergata, Roma, Italy.

In previous in vitro studies we found that contact between mouse primordial germ cells and other cell types (neighbouring somatic cells or established TM4 or STO cell lines) is crucial for supporting primordial germ cell survival and proliferation and for activating their motility. We have studied primordial germ cell adhesion to different cell monolayers (STO, TM4, COS and F9 cells) as an in vitro model for interactions between primordial germ cells and cellular substrates. The results suggest that these cell interactions are mediated by multiple mechanisms involving Steel factor and its receptor encoded by c-kit, carbohydrates and possibly other unknown factors. We find that Steel factor and leukaemia inhibitory factor are survival rather than proliferation factors for primordial germ cells. Both molecules prevent primordial germ cell death in culture by suppressing apoptosis. Morphological and molecular features of primordial germ cell apoptosis in vitro are reported. Activation of protein kinase C does not promote primordial germ cell proliferation, but compounds known to enhance intracellular levels of cAMP (i.e. dibutyryl cAMP and forskolin) markedly stimulate primordial germ cells to proliferate in culture. We have preliminary results indicating that neuropeptides PACAP-27 and PACAP-28 are possible physiological activators of adenylate cyclase in primordial germ cells.

## Publication Types:

- Review
- Review, Tutorial

PMID: 7530618 [PubMed - indexed for MEDLINE]



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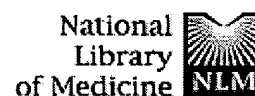
Full Text @ Cell Press

**Derivation of pluripotential embryonic stem cells from murine primordial germ cells in culture.****Matsui Y, Zsebo K, Hogan BL.**Department of Cell Biology Vanderbilt University Medical School  
Nashville, Tennessee 37232.

Steel factor (SF) and LIF (leukemia inhibitory factor) synergistically promote the proliferation and survival of mouse primordial germ cells (PGCs), but only for a limited time period in culture. We show here that addition of bFGF to cultures in the presence of membrane-associated SF and LIF enhances the growth of PGCs and allows their continued proliferation beyond the time when they normally stop dividing in vivo. They form colonies of densely packed, alkaline phosphatase-positive, SSEA-1-positive cells resembling undifferentiated embryonic stem (ES) cells in morphology. These cultures can be maintained on feeder layers for at least 20 passages, and under appropriate conditions give rise to embryoid bodies and to multiple differentiated cell phenotypes in monolayer culture and in tumors in nude mice. PGC-derived ES cells can also contribute to chimeras when injected into host blastocysts. The long-term culture of PGCs and their reprogramming to pluripotential ES cells has important implications for germ cell biology and the induction of teratocarcinomas.

PMID: 1381289 [PubMed - indexed for MEDLINE]

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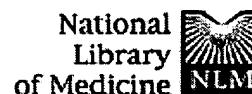
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FULL-TEXT ARTICLE****In vitro survival and proliferation of porcine primordial germ cells.****Shim H, Anderson GB.**

Department of Animal Science, University of California, Davis 95616, USA.

Primordial germ cells (PGC) collected from the genital ridge of Day 25 porcine embryos were cultured on STO feeder cells in medium with or without supplemented growth factors. The effects on porcine PGC proliferation of leukemia inhibitory factor (LIF), LIF + stem cell factor (SCF) or LIF + SCF + basic fibroblast growth factor (bFGF), growth factors shown to be essential for in vitro survival and proliferation of murine PGC, were tested. After histochemical staining, both freshly collected and cultured PGC expressed alkaline phosphatase activity. With or without supplemented growth factors, porcine PGC survived and proliferated in culture for at least 5 d. None of the growth factors tested markedly enhanced in vitro growth of porcine PGC. These results suggest that growth factors provided by either the STO feeder layer or the cultured PGC themselves are sufficient to support in vitro survival and proliferation of porcine PGC. With the support of STO cells, addition of growth factors shown to be essential for the in vitro growth of murine PGC is not required for survival and proliferation of cultured porcine PGC.

PMID: 10732031 [PubMed - indexed for MEDLINE]



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NEW Books

**The membrane-bound isoform of stem cell factor synergizes with soluble flt3 ligand in supporting early hematopoietic cells in long-term cultures of normal and aplastic anemia bone marrow.****Slanicka Krieger M, Nissen C, Manz CY, Toksoz D, Lyman SD, Wodnar-Filipowicz A.**

Research Department, University Hospital Basel, Switzerland.

The hematopoietic growth factors stem cell factor (SCF) and flt3 ligand (flt3L) are produced within the hematopoietic microenvironment in a membrane-bound and soluble isoform. To elucidate the relevance of the two isoforms in the network of early-acting cytokines, we examined the interaction of membrane-bound SCF with the soluble forms of SCF and flt3L in long-term cultures of human bone marrow cells. Feeder layers of the murine SCF-deficient Steel stromal cell line transfected with human cDNA stably expressing SCF as a transmembrane molecule were used to support growth of mononuclear cells and CD34+ progenitors derived from normal human bone marrow or from hypoplastic marrow of patients with aplastic anemia (AA). The output of nonadherent progenitor cells representing colony-forming units (CFU) and high-proliferative potential colony-forming cells (HPP-CFC) was scored weekly in secondary methylcellulose cultures; the number of colonies derived from long-term culture-initiating cells (LTC-IC) was determined in nonadherent and adherent cells at 5 weeks. Membrane-bound SCF expressed in the stromal layer was more effective than soluble SCF and soluble flt3L in maintaining clonogenic progenitors. Furthermore, the transmembrane form of SCF effectively synergized with both exogenously supplied factors, although the effect of flt3L was superior to the effect of soluble SCF. In cultures of normal bone marrow cells, addition of flt3L enhanced the total number of CFU and HPP-CFC-type progenitors, primarily of the granulocyte/macrophage lineage, by six- to ninefold after 3 weeks and of LTC-IC-derived colonies by 13-fold after 5 weeks of culture. In cultures of AA cells, both the number and the survival rate of clonogenic precursors were severely impaired even in the presence of flt3L, which,

however, yielded a two- to sixfold enhancement of CFU and HPP-CFC numbers at 1 to 2 weeks. In comparison with the hematopoietic function of human Dexter-type stroma cultures, murine feeders expressing high levels of membrane-associated human SCF were equivalent in supporting hematopoiesis during the initial 3 to 4 culture weeks when supplemented with flt3L. These results demonstrate that soluble flt3L interacts with membrane-bound SCF in supporting the long-term growth of bone marrow progenitor cells. The hypothesis that SCF and flt3L function synergistically during the very early stages of human hematopoiesis is thereby reinforced.

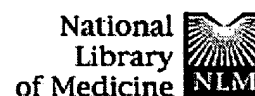
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NEW Books

**Primary culture of porcine PGCs requires LIF and porcine membrane-bound stem cell factor.****Durcova-Hills G, Prella K, Muller S, Stojkovic M, Motlik J, Wolf E, Brem G.**

Institute of Animal Physiology and Genetics, Libechov, Czech Republic.

We studied the effect of murine leukaemia inhibitory factor (LIF), human basic fibroblast growth factor (bFGF) and porcine stem cell factor (SCF) on the survival and/or proliferation of porcine primordial germ cells (PGCs) obtained from 27-day-old embryos in vitro. PGCs were cultured in embryonic stem cell (ESC) medium supplemented with or without either LIF (1000 IU/ml) alone or LIF together with bFGF (10 ng/ml). They were seeded on mitotically inactivated feeder cells, either STO or transfected STO cells (STO#8), expressing the membrane-bound form of porcine SCF. PGCs were identified by their alkaline phosphatase (AP) activity and counted after 1, 3 and 5 days in culture. After 1 day of culture, PGCs cultured on STO#8 cells showed significantly higher survival than PGCs cultured on STO cells ( $p < 0.05$ ). The combined effect of SCF and LIF caused a significant increase in PGC number by day 3 of culture when PGCs were cultured on either STO cells ( $p < 0.01$ ) or STO#8 ( $p < 0.001$ ). When SCF and LIF were used together with bFGF no increase in the PGC number was observed. Our results suggest that the membrane-bound form of porcine SCF plays a pivotal role in the primary culture of porcine PGCs and that bFGF is not required in vitro.

PMID: 9854799 [PubMed - indexed for MEDLINE]

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